PHARMACOLOGICAL REVIEWS
Vol. 18, No. 1, Part I
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Printed in U.S.A.

P. INTERRELATIONSHIPS OF CATECHOLAMINES WITH OTHER ENDOCRINE SYSTEMS

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The responses of mammalian organisms to environmental stimuli are mediated through changes in the activity of enzyme systems. Such changes occur over periods of time, ranging from hours to days and even months, but many occur instantaneously. Until recently, biologists have been concerned mainly with enzymes which are adapted slowly by processes that alter the enzyme-forming systems. But many responses are mediated by the nervous system, and great interest has been aroused in the nature of the mechanisms by which certain neurohormones activate enzymes almost instantaneously.

The instantaneous activation of enzymes is particularly important in considering the fluctuating energy requirements of the body. Since the adrenergic system is spread diffusely throughout the brain and links up with a host of somatic functions, behavioral patterns that require instant bursts of energy are assured of sufficient metabolic fuel through signals along sympathetic nerves. Catecholamines, released by these signals, trigger the activation of adipose tissue lipase, thus causing the breakdown of triglyceride to free fatty acid (FFA) (22), and of muscle and liver phosphorylase, which catalyzes the breakdown of glycogen to glucose-1-phosphate or glucose (30).

The unique role of the sympathetic system in controlling rapid changes in the mobilization of energy fuel by glycogenolysis and lipolysis has been obscured by the fact that a number of polypeptides can also evoke a rapid stimulation of these processes (25).

Despite the facts that adipose tissue is innervated by sympathetic nerves and that norepinephrine (NE) is present in adipose tissue in amounts far in excess of those needed for circulatory function (19), much discussion is still centered on whether a rapid mobilization of metabolic fuel is evoked under physiological conditions by hormones other than catecholamines released from the sympathetic system. Techniques of total block of adrenergic function have made it possible to determine the extent to which the sympathetic system is responsible for augmenting the output of energy substrates. The block is produced by depletion of peripheral stores of catecholamines, or by preventing their physiological release. Either of two procedures is now used in this laboratory: a) adrenal demedullation followed by depletion of peripheral NE stores by means of two nonsedative doses of the reserpine analogue, syrosingopine; b) adrenal demedullation followed by treatment with the bretylium-like compound, BW 392C60, which prevents nerve impulses from releasing NE in response to sympathetic stimulation (8).

PHYSIOLOGICAL ROLE OF THE SYMPATHETIC SYSTEM IN MOBILIZING ENERGY SUBSTRATES

Cold-exposure. When normal rats are placed in a cold room at 4°C, their metabolism increases from about 1350 cal. per hr to about double this value. Accompanying the rise in metabolism is an elevation in the activity of adipose tissue lipase. This in turn causes an increased mobilization of FFA, a 2-fold elevation in plasma FFA and a rise in the concentration of liver and plasma triglycerides. At the same time, plasma glucose levels are increased about 70% (11).

When adrenergic nerve function has been blocked as described above, the basal metabolism, and the plasma FFA and glucose levels of the rats are almost normal. On exposure to cold, however, these rats can neither conserve heat nor generate additional heat; they show no signs of vasoconstriction, piloerection or shivering (11). Body temperature rapidly declines and heat is lost at the rate of about 1400 cal. per hr, equivalent to the increase in the metabolism of the control rats at 4°C. As a result the rats die in 3 hr at a body temperature of about 15°C. In the cold these rats do not produce the expected increases in plasma FFA and glucose nor in liver and plasma triglycerides. This breakdown in the supply of fuel results from an impairment of the mechanisms that activate adipose tissue lipase (14) and perhaps skeletal muscle and liver phosphorylase.

Although these rats are unable to mobilize additional energy substrates, they still hypermobilize ACTH as shown by the plasma corticosterone, which reaches a concentration of 50 μg per 100 ml of plasma, the steady-state level corresponding to the rate of maximal synthesis (15). This result is of considerable significance since a number of workers have postulated that a physiological role of ACTH in mobilizing FFA from adipose tissue is comparable to that of glucagon in mobilizing glucose from liver. These results indicate that the secretion of ACTH, even under condition of profound physiological "stress," is not sufficient to stimulate the release of FFA.

The objection could be raised that either of the drugs used to produce functional sympathectomy might affect glycogenolysis and lipolysis by some other action. Accordingly, sympathetic function is also suppressed by giving the ganglionic blocking agent, chlorisondamine (Ecolid), to adrenal-demedullated rats. On exposure to cold, lipase activity is not increased, plasma FFA and glucose do not rise, and piloerection, vasoconstriction as well as shivering are prevented; as a consequence the animals lose 1000 cal. per hr and die in about 5 hr (3).

To show that the effects of the block by BW 392C60, syrosingopine or chlorisondamine are attributable to lack of peripheral catecholamines, the rats are treated with epinephrine-in-oil and then exposed to cold. The animals now show an increase in plasma levels of FFA and glucose, exhibit piloerection, vasoconstriction and shivering, and maintain a normal body temperature. With repeated doses of the catecholamine, the survival time may be extended for 24 hr or more.

When the organism is faced with a sudden need for additional body fuels, to what extent is this dealt with by the peripheral sympathetic nerves rather than the adrenal medulla? When adrenal-demedullated rats are subjected to cold, the level of plasma FFA is doubled. In addition the animals show vasoconstriction,

piloerection and shivering. Thus circulating epinephrine is not an absolute requirement for shivering. The plasma glucose level, however, is not raised (14), and the body temperature slowly declines with a net loss of heat of about 400 cal. per hr. This continuous drain of body heat ends in death of animals in about 12 hr at a body temperature of about 15°C. If the demedullated rats are given injections of epinephrine-in-oil and then exposed to cold, the plasma levels of glucose as well as FFA are elevated and the body temperature does not decline (14).

These results imply that the emergency mobilization of FFA from fat depots is under the precise and direct control of NE released from sympathetic nerves, and that epinephrine from the adrenal medulla is not needed for this function. Apparently, maximal lipolysis is elicited by a small increase in sympathetic tone so that epinephrine can produce no additional effect on the lipolytic system; thus the mild exercising of adrenal-demedullated rats in a treadmill results in a marked rise in plasma FFA, but no rise in plasma glucose (16). These results suggest that moderate exercise may be fueled by an increased release of FFA through the action of NE.

In view of the marked lipolytic action of NE as well as the ability of demedullated animals to perform moderate exercise, it is of interest that triglycerides as well as glycogen are available in storage depots localized in skeletal muscle. Present evidence indicates that triglyceride stores in skeletal muscle fibers act as adipose tissue depots in miniature, subject to the same regulatory mechanisms as adipose tissue itself (2). According to this view, triglycerides in skeletal muscle constitute stores of energy that are readily available to muscle fibers and are ultimately replenished by fatty acids transported from adipose tissue. In this way the central nervous system assures the availability of energy substrates for local needs in the very tissues through which the behavioral responses are channeled.

Muscular exercise. Shivering requires two signals, a cholinergic one causing muscle fibers to contract and an adrenergic one, probably supplying fuel for muscle work. Is the sympathetic system needed also to provide metabolic fuel for the normal activity of skeletal muscle? Normal rats forced to swim with weights attached to their tails are able to mobilize glucose and FFA and swim for about 5 min; but animals whose adrenergic nerve function has been blocked are not able to mobilize additional energy substrates and stop swimming in about half this time (3). Similarly, when these rats are forced to exercise on a treadmill, they soon collapse from exhaustion (27). They cannot utilize skeletal muscle glycogen or increase plasma glucose and FFA levels.

Drugs. Many drugs in large doses increase the output of FFA presumably by a central action on the sympathetic outflow. Among these are alcohol and morphine. The output of FFA may also be stimulated peripherally by the ganglionic stimulant, 1,1-dimethyl-4-phenylpiperazinium iodide. In rats whose adrenergic nerve function is blocked, the FFA output is not increased by these drugs (3, 13).

The failure of these rats to cope with an increased need for energy indicates that the sympathetic nervous system is essential for the survival of the mam-

malian organism, at least in the case of the rat. This conclusion is counter to the general belief that the sympathetic system is useful but not essential for survival, a view based upon the observation of Cannon *et al.* (7) that when surgically sympathectomized cats are exposed to cold they maintain an almost normal body temperature by immediate and violent shivering. The intensive surgical sympathectomy described by Cannon is now known to be incomplete since the so-called intermediate ganglia escape removal by his technique (17). The metabolic effects of the adrenergic system can be blocked in cats by the ganglionic blocking agent chlorisondamine or by N-isopropylmethoxamine (4), a β-adrenergic blocking agent that prevents catecholamines from mobilizing FFA and glucose. When cats so pretreated are exposed to cold they do not shiver nor show piloerection; consequently the body temperature declines by about 6°C over a period of 2 or 3 hr (3). For an animal the size of a cat this represents a sizeable loss of heat. In contrast, the temperature of control animals does not fall; they exhibit piloerection and occasional bursts of shivering. These results are a further indication that the sympathetic system is indispensable in responding to rapid changes in energy requirements.

An important outgrowth of these studies is the recognition that animals will not shiver without an intact sympathetic nervous system. Shivering is thus an adrenergic as well as a cholinergic function. In fact shivering may be blocked by intercepting the motor signal (curare) or by suppressing the adrenergic signal.

INTERACTION OF ADRENAL CORTEX AND SYMPATHETIC NERVOUS SYSTEM

Rats whose adrenergic nerve function has been blocked are remarkably similar to adrenalectomized rats in their responses to external stimuli that require an increased expenditure of energy. The basal metabolism and plasma FFA and glucose levels are almost normal, but on exposure to cold neither animal can conserve heat nor generate additional heat; they show no sign of shivering, piloerection or vasoconstriction, and are unable to mobilize extra FFA or glucose or to elevate liver and plasma triglycerides. The body temperature of the adrenalectomized rat also declines rapidly, heat is lost at a rate of about 900 cal. per hr and the animals die in about 6 hr. It is of considerable interest that the adrenalectomized rats live longer in the cold than those lacking adrenergic nerve function (3). In forced-swimming studies, neither animal is able to mobilize fatty acids and glucose or to swim for more than a short time. Moreover, when forced to exercise in a treadmill, both animals soon collapse from exhaustion and fail to mobilize additional energy substrates in appreciable amounts (27).

Because of the lack of sympathetic responses in the adrenalectomized rats we thought that the aforementioned effects might result from loss of peripheral catecholamines. But treatment of adrenalectomized rats with epinephrine and then exposing them to cold fails to restore the lipolytic and glycogenolytic responses or to affect the survival of the animals. In fact, the catecholamine exerts an adverse effect, for the animals die sooner in the cold. The effects of adrenalectomy may be ascribed to the lack of adrenal corticoids since treatment of adrenalectomized rats with cortisone or prednisolone 3 hr before cold exposure

restores the lipolytic and glycogenolytic responses. In addition the animal displays piloerection, vasoconstriction, shivering and a normal body temperature.

The possibility was raised that in the absence of adrenal cortical steroids the catecholamine cannot transmit its usual signals. This is evident from experiments which show that the injection of epinephrine-in-oil into adrenalectomized rats at room temperature does not elevate plasma FFA. Epinephrine still elicits some increase in plasma glucose though the response is relatively small compared to that seen in normal rats. On treatment of the adrenalectomized animals with glucocorticoids, the lipolytic and glycogenolytic responses to catecholamines are completely restored.

The importance of the corticoids in the expression of the sympathetic nervous system is also evident by experiments with certain drugs. For example, in adrenalectomized rats large doses of ethanol or morphine no longer increase the levels of plasma FFA and liver triglycerides, and the ganglionic stimulating agent, dimethylphenylpiperazinium, no longer increases plasma FFA (3, 13). Again, pretreatment with cortisone restores the lipolytic effects (13).

It is not easy to see why the removal of either catecholamines or adrenal cortical hormones from the organism should impair sympathetic responses, including those involved in the quick mobilization of extra energy substrates. The realization that lipolysis and glycogenolysis cannot be stimulated suggests that cortisone and catecholamines act on some common pathway and that elucidation of the biochemical lesion in adrenalectomy might help in disclosing the mechanism by which catecholamines instantaneously activate these processes.

Since adrenalectomized rats can be sustained for some time on an adequate intake of saline provided they are not exposed to situations requiring extra metabolic energy, the possibility was entertained that changes in electrolytes are involved in the supportive action of the corticosteroids. If so, an adrenalectomized rat maintained on saline would not be functionally adrenalectomized. This view was tested by maintaining adrenalectomized rats on tap water instead of saline before exposing them to cold. The longer the animals are deprived of salt, the more quickly does body temperature decline: rats maintained on saline until exposed to cold survive 6 hr; animals deprived of saline for 2 days and then placed in the cold survive only about 3.5 hr. These animals now lack completely the ability to mobilize extra energy substrates and to conserve body heat. The net heat loss is about 1400 cal. per hr, *a rate similar to that seen after block of adrenergic function.* Epinephrine elicits no rise whatsoever in plasma glucose or FFA. The fact that the lack of salt exacerbates the effects of corticoid insufficiency on the sympathetic system encourages the suggestion that shifts in electrolytes are responsible in some way for the inability of adrenalectomized rats to respond to situations requiring an increased output of energy. Thus adrenalectomized animals, exposed to cold after 1 to 2 days without saline, display no piloerection, vasoconstriction, or shivering. Since even exogenous catecholamines do not increase levels of FFA or glucose it may be inferred that the adrenergic receptors which account for the adaptive responses to cold are no longer responsive.

To circumvent the possibility that adrenalectomized rats react abnormally to various stimuli merely because of circulatory failure associated with the loss of fluid and electrolytes, studies were performed using epididymal fat pads *in vitro*. The adipose tissue from adrenalectomized animals still mobilizes some FFA, but in confirmation of the work of others (21, 26) the response to catecholamines is greatly diminished. Cortisone added to the incubation mixture does not restore the lipolytic response, but the fat pad again reacts to catecholamines if the rats are pretreated with cortisone.

The relative effectiveness of aldosterone and cortisone in protecting adrenalectomized rats against cold exposure were compared. A single dose of cortisone or prednisolone exerts a considerable protective effect upon adrenalectomized rats that have been deprived of salt for 2 days. Aldosterone is also protective but only if administered in daily doses from the time the animals are deprived of saline. This suggests that glucocorticoids might affect tissue electrolytes at the cellular level, whereas the main action of aldosterone is on the kidney. This view is in accord with that of Swingle *et al.* (29), who reported that adrenalectomized dogs in which electroshock had produced circulatory failure are restored to vigor and normal activity by a single dose of prednisolone despite low levels of plasma Na and Cl. In contrast, desoxycorticosterone and aldosterone in doses that increase the levels of plasma Na and Cl do not counteract the fall in plasma volume and blood pressure. These workers concluded that mineralocorticoids act primarily on the kidney to maintain the external electrolyte balance, while glucocorticoids bring about dramatic and rapid recovery by shifting fluid and electrolytes between the intra- and extracellular compartments. They also suggested that glucocorticoids are a vital part of the mechanism which maintains the internal water and electrolyte balance of vertebrate organisms.

Thus it is not unreasonable to propose that the sympathetic nervous system fails in adrenal corticoid insufficiency because of electrolyte imbalance. On this basis, the lethal effect of cold in adrenalectomized rats may be attributed to the poor responsiveness of adrenergic receptors to catecholamines. This interaction of the adrenal corticoids with the sympathetic nervous system throws some light on certain ambiguous aspects of glucocorticoid actions. These are the actions which, according to Ingle (12), can be described in terms of not what they do themselves, but what they do to responses of other physiological systems. Ingle suggested that the adrenal steroids support the "normality" of tissue responses to stimuli which arise from noncortical systems, and used the term "permissive" to describe these effects. According to our results this action of the adrenal corticoids permits the sympathetic receptors to respond to catecholamines.

Incidentally these findings might have important implications in the problems of shock since they point out the difficulty of separating adrenal corticoid insufficiency from sympathetic failure. It is possible that in some forms of shock the sympathetic receptors are simply unable to respond to catecholamines. In such situations the administration of catecholamines might actually worsen the condition.

INTERACTION OF SYMPATHETIC AND ADRENOCORTICAL SYSTEMS ON ENZYMES THAT MOBILIZE ENERGY FUEL

In physiology the concept of the receptor is used to describe a hypothetical substance in or on the cell membrane, which combines with a neurohormone to modify cellular function. For example, the response of adipose tissue to NE is through the activation of adipose tissue lipase, but the intervening events between receptor-NE interaction and the activation of lipase are unknown. To bridge biochemistry with physiology, we must know the nature of these receptors and how they interact with neurohormones. In this regard it is difficult to overestimate the importance of the work of Sutherland and co-workers (30) which has given rise to the concept of a trigger substance, cyclic 3′,5′-AMP, formed in the target cells by the action of catecholamines. This nucleotide is a biochemical trigger through which a physiologic event—the release of a neurohormone —is translated to a biochemical event—glycogenolysis.

Since both sympathectomy and adrenalectomy impair processes that enable the organism to increase the mobilization of FFA and glucose, we have investigated the nature of the biochemical lesion in adrenalectomy, in the hope of disclosing the mechanism by which catecholamines produce instantaneous activation of these processes.

A clue to the mechanism by which catecholamines activate skeletal muscle phosphorylase *in vivo* is provided by comparison of the response of the enzyme to epinephrine in adrenalectomized rats maintained with and without saline. When the rats are maintained on saline, about 45% of the enzyme is activated by epinephrine—a reflection perhaps of the "adrenocortical" effects of saline. In contrast, when adrenalectomized rats are deprived of saline for 2 days, muscle phosphorylase is unresponsive to epinephrine. If adrenalectomized rats are given repeated doses of cortisone during 2 days of salt deprivation, epinephrine almost completely activates phosphorylase. Adrenalectomized rats maintained on aldosterone or desoxycorticosterone also show activation of phosphorylase in response to epinephrine (31). The effects of these mineralocorticoids raise the possibility that activation of the enzyme by epinephrine requires a certain electrolyte environment. Support for this possibility is found in requirements *in vitro* showing that the degree to which phosphorylase in muscle is activated by NE is dependent on the ratio of extracellular K/Na. In contrast, the activation of phosphorylase by cyclic 3′,5′-AMP is not dependent on the ionic environment (31). Although it is by no means proved that electrolyte imbalance is the cause of sympathetic failure in adrenalectomized rats, experimentally it seems a worthwhile postulate.

An additional clue to the nature of the biochemical lesion in adrenalectomy is disclosed by the finding that both glucagon and epinephrine fail to increase plasma glucose levels in adrenalectomized rats. Treatment of these rats with a glucocorticoid restores the responses to epinephrine and glucagon. Thus two hormones, both of which normally act through cyclic 3′,5′-AMP, do not exert an effect in adrenalectomized rats. Similarly the increased lipolysis elicited by

ACTH is not produced in adipose tissue from adrenalectomized rats; again the response is restored by pretreatment of the rat with a glucocorticoid. These results strongly suggest that the lesion in adrenalectomy lies at a step between the release of the adrenergic neurohormone and the formation of cyclic 3′,5′-AMP.

A further indication of the nature of the biochemical lesion in adrenalectomy was obtained by determining the effects of epinephrine on the various enzymes required to activate the phosphorylase system. After the injection of epinephrine, the phosphorylase *b* kinase system extracted from the skeletal muscle of normal rats stimulates the conversion of a purified preparation of phosphorylase *b* to the active *a* form; in contrast, the kinase system from adrenalectomized rats is unresponsive. On pretreatment of rats with cortisone, the responsiveness of the kinase to epinephrine is restored. These results suggest that the biochemical lesion in adrenalectomized rats involves failure of epinephrine to stimulate the formation of cyclic 3′,5′-AMP. In accord with this view, cyclic 3′,5′-AMP added to the muscle extract of either normal or adrenalectomized rats activates the kinase system. These results strongly suggest that the adrenalectomized rats are unable to form cyclic 3′,5′-AMP. The proof of this hypothesis was demonstrated by comparing the effects of epinephrine on the tissue concentrations of cyclic 3′,5′-AMP on normal and adrenalectomized rats. The result was that epinephrine does not elevate the levels of this nucleotide in adrenalectomized rats (31).

These experiments fix the lesion at the step lying between the release of catecholamine and the activation of adenyl cyclase. Since epinephrine activates adenyl cyclase in homogenates (18), it is possible that the action of the corticoids is a membrane effect which permits catecholamines to reach the enzyme.

THEOPHYLLINE AS A TOOL IN STUDIES OF ADIPOSE TISSUE LIPOLYSIS

Since the catecholamines NE, epinephrine, and isoproterenol, and the polypeptide corticotropin (ACTH), added to adipose tissue *in vitro* all stimulate lipolysis to the same degree, it is generally assumed that the rate of this process is limited by the amount of lipase in adipose tissue. From the studies described below it is evident that normally the lipase is only partly activated by an excess of catecholamines and that the limitation in the activity of the enzyme is the steady-state level of cyclic 3′,5′-AMP.

A few months ago we "rediscovered" theophylline, a substance reported by Butcher and Sutherland (5) to inhibit phosphodiesterase, the enzyme that inactivates cyclic 3′,5′-AMP. Quite by accident, we found that the injection of theophylline into rats produces a prolonged elevation in the concentration of plasma FFA. The concentration rises slowly, about 45 min being required to reach a plateau. These results suggested that cyclic 3′,5′-AMP is involved in lipolysis, and that the increased level of FFA is mediated through the gradual accumulation of cyclic 3′,5′-AMP. Furthermore, since the rise in FFA was considerably higher than that produced by catecholamines, the possibility was considered that the lipase content of adipose tissue is higher than heretofore considered. The response to theophylline is dose-dependent (table 1) and, after large doses of the

TABLE 1

Effects of theophylline on plasma FFA and glucose levels

Theophylline	FFA	Glucose
mg/kg,i.p.	*μEq/ml*	*mg %*
	0.19	113
18	0.49	112
60	0.72	116
180	1.08	160

FFA and glucose assayed in plasma from blood taken 30 min after injection of theophylline.

TABLE 2

Effects of theophylline and NE on plasma FFA

Treatment	FFA	
	μEq/ml	Δ due to treatment
None	0.23	
NE (0.2 mg/kg, i.m.)	0.63	0.40
Theophylline (60 mg/kg, i.p.)	0.45	0.22
NE (0.2 mg/kg, i.m.) plus theophylline (60 mg/kg, i.p.)	1.18	0.95

FFA assayed in plasma from blood taken 30 min after NE and 60 min after theophylline injection.

drug, the FFA concentration reaches a level about two times higher than the maximum concentration elicited by NE. These doses of theophylline also elevate the level of plasma glucose by 50%. If theophylline interferes with the inactivation of cyclic 3′,5′-AMP, and NE increases its synthesis, the combined effects of the agents should be more than additive. In accord with this view, NE and theophylline together elicit more than an additive rise in plasma FFA (table 2).

The question arises whether theophylline will induce lipolytic effects in animals deprived of adrenergic function. The administration of theophylline to these rats elicits the same rise in plasma FFA as in the controls. This indicates that cyclic AMP is formed even in the absence of sympathetic tone. Moreover, theophylline exerts a similar effect in adrenalectomized rats.

The possibility that cyclic 3′,5′-AMP is directly involved in lipolysis was studied by examining the effects of theophylline in adipose tissue *in vitro*. The lipolytic response, as measured by glycerol output, is dependent on the concentration of theophylline and is maximal at a concentration of about 5×10^{-3} M (fig. 1, left). This response is three times greater than the maximal response yielded by NE (fig. 1, right). These results indicate that the lipase in adipose tissue is only partially activated by NE, and that when adenyl cyclase is fully activated by NE, the rate of lipolysis is still limited by the concentration of cyclic 3′,5′-AMP. In agreement with this view, the effects of NE and theophyl-

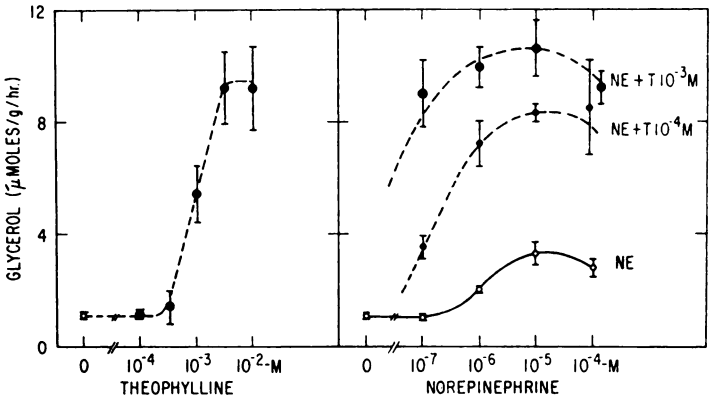


FIG. 1. Left, lipolytic effects of theophylline in adipose tissue; right, lipolytic effects of NE and of combinations of NE and theophylline (T).

line are more than additive *in vitro* (fig. 1, right). Theophylline, in an amount that exerts no lipolysis by itself (10^{-4} M) is able to enhance the lipolytic effect of a wide range of NE concentrations, and these responses are far greater than those elicited by maximally effective doses of NE. When the concentration of theophylline is raised to 10^{-3} M, the lipolytic rate is further enhanced until lipase is completely activated. As a result, the highest lipolytic activity elicited by the drug combination equals but does not exceed the maximal activity elicited by high concentrations of theophylline alone. From these results it may be inferred that the activation of lipase by catecholamines is normally limited by the steady-state concentration of cyclic 3′,5′-AMP and that theophylline, by blocking phosphodiesterase causes cyclic 3′,5′-AMP to accumulate to a concentration that causes complete activation of lipase.

If ACTH also acts through cyclic 3′,5′-AMP, the lipolytic action of this hormone would also be enhanced by theophylline. Figure 2 compares the effects of

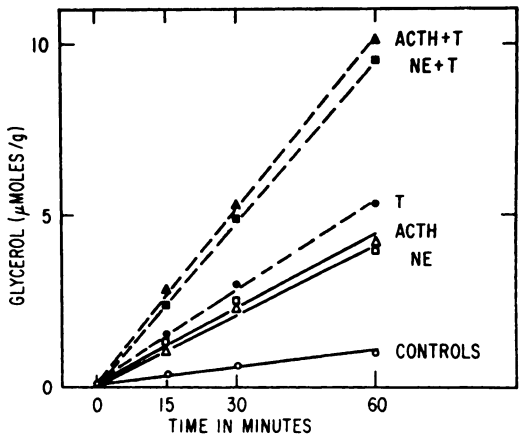


FIG. 2. Lipolytic effects of NE or ACTH and of combinations with theophylline (T)

theophylline (10^{-3} M) on the lipolytic activity of equiactive concentrations of NE and ACTH. Since the lipolytic effects of both hormones are affected to the same extent, it may be inferred that the lipolytic effect of ACTH is mediated through cyclic 3′,5′-AMP.

The data in table 3, in accord with those of Butcher *et al.* (6), show that an increased lipolysis is associated with an elevated level of cyclic 3′,5′-AMP. Thus the lipolytic activity and the steady-state level of cyclic 3′,5′-AMP are both markedly increased in the presence of high concentrations of theophylline. At these concentrations the drug inhibits adipose tissue phosphodiesterase by about 85%.

Thus far our evidence showing that the lipolytic effects of catecholamines are mediated through cyclic 3′,5′-AMP has been indirect, since we have not shown that the nucleotide is actually the cause of the lipolysis. Rizack (23) has shown that cyclic 3′,5′-AMP activates a partially purified preparation of lipase but this has not been demonstrated for the whole cell. We have studied the effects of cyclic 3′,5′-AMP on fat cells prepared according to Rodbell (24). As shown in table 4, the nucleotide by itself has only a negligible effect on the lipolytic activity

TABLE 3

Effects of theophylline on glycerol release, cyclic 3′,5′-AMP level, and blockade of phosphodiesterase

Concentration of Theophylline	Cyclic AMP	Glycerol	% Inhibition of Phosphodiesterase[a]
	μmoles/g	*μmoles/g/hr*	
	0.0005	0.68	0
1×10^{-5} M	0.0005	0.68	16
5.6×10^{-3} M	0.0022	4.1	82
1×10^{-2} M	0.0027	5.5	86

[a] Phosphodiesterase assay is essentially that described by Butcher and Sutherland (5) except that H^3-cyclic 3′,5′-AMP is used at 10^{-7} M and adenosine is measured as the end product.

TABLE 4

Effects of theophylline and cyclic 3′,5′-AMP on the glycerol output of fat cells

Additions	Glycerol
	μmoles/mmole triglyceride/hr
None	0.2
Theophylline (4×10^{-4} M)	4.5
Cyclic 3′,5′-AMP (5×10^{-3} M)	1.4
Cyclic 3′,5′-AMP (1×10^{-2} M)	6.6
Theophylline (4×10^{-4} M) + cyclic 3′,5′-AMP (5×10^{-3} M)	17
Theophylline (4×10^{-4} M) + cyclic 3′,5′-AMP (1×10^{-2} M)	42
Theophylline (1×10^{-2} M)	38
Theophylline (1×10^{-2} M) + cyclic 3′,5′-AMP (4×10^{-5} M to 1×10^{-2} M)	38

of fat cells presumably because the small amounts that enter these cells are quickly inactivated. On the addition of theophylline in an amount that has only slight activity by itself, the cyclic 3′,5′-AMP that enters cells is in part protected and can now elicit a maximal activation of the lipase. The same table shows that the lipolytic activity of theophylline in amounts that produce a maximum effect are not increased by cyclic 3′,5′-AMP in levels ranging from 4×10^{-5} M to 10^{-2} M. This provides additional proof that a maximal lipolytic action is reached when adipose tissue lipase is completely activated.

The rate of cyclic 3′,5′-AMP turnover may be calculated from the rate of accumulation of the nucleotide after incubation of rat epididymal fat pads with theophylline. Preliminary results show that the rate is about 7 μg per g per hr in the normal unstimulated fat pad and about 15 μg per g per hr after stimulation by NE.

ADRENERGIC BLOCKING AGENTS

Both *alpha*- and *beta*-adrenergic blocking agents are able to inhibit the lipolytic effects of catecholamines, but the *beta* blockers are much more potent in this regard.

The use of theophylline as an inhibitor of cyclic 3′,5′-AMP inactivation permits the action of *alpha* and *beta* blocking agents to be compared. *In vitro* studies show that the *beta* blocking agent dichloroisoproterenol (DCI) at a concentration of 5×10^{-5} M inhibits the lipolytic effect of NE by 50%, but has little or no action on the effect of theophylline; however, very high concentrations of DCI (180×10^{-5} M) also inhibit the action of theophylline. In contrast, phentolamine, an *alpha* blocking agent, inhibits the lipolytic effects of NE and theophylline to the same extent but high concentrations of the drug (65×10^{-5} M) are needed. These results suggest that, in contrast to DCI, which blocks competitively the lipolysis produced by NE, phentolamine blocks the effects of NE by acting on some step subsequent to the formation of cyclic 3′,5′-AMP. In high concentrations, the *beta* blocking agent might also act at this step since it inhibits the action of theophylline.

INTERACTION OF THYROID AND SYMPATHETIC SYSTEMS

Many of the manifestations of hyperthyroidism, including enhanced responses to external stimuli and elevation of basal metabolic rate, are reminiscent of increased sympathetic activity, and the possibility has been raised that the action of thyroid hormones is mediated through catecholamines. Furthermore, hypothyroidism is associated with a poor response to catecholamines (28). A recent proposal that the thyroid hormones increase the rate of NE synthesis seems unlikely since the turnover rate of cardiac NE is similar in normal and hyperthyroid rats (1).

Published reports show that the epididymal fat pads from hypothyroid rats are poorly responsive to the action of epinephrine; conversely, adipose tissue from hyperthyroid rats shows an exaggerated lipolytic response to catecholamines (9, 10). Recent work also indicates that thyroid hormones have a primary function in regulating protein synthesis (32, 33). The following studies (unpublished)

indicate that thyroid hormones increase the amounts of adenyl cyclase in adipose tissue. As a result the same concentration of catecholamines can stimulate the formation of greater amounts of cyclic 3′,5′-AMP.

THE EFFECTS OF TREATMENT WITH THYROXIN

Rats were made hyperthyroid by the administration of thyroxin (1 mg/kg) for 5 days. The basal metabolic rate increases by about 40% and the protein content of adipose tissue increases by 15%. Adipose tissue from these rats is more sensitive to theophylline than that from the untreated animals (fig. 3, left). This suggests that thyroxin treatment may have increased the quantity of adenyl cyclase in this tissue. NE in various concentrations incubated with adipose tissue from these animals enhances the lipolytic response almost three times relative to the controls (fig. 3, right). Since NE is added in amounts sufficient to activate adenyl cyclase completely, it may be inferred that the formation of this enzyme has been increased by the action of thyroxin. Further evidence of this is seen from the lipolytic effects of various combinations of NE and theophylline. The NE-induced lipolysis in adipose tissue from normal and hyperthyroid rats is increased by the action of theophylline. Although the adipose tissue from the hyperthyroid rats is more sensitive to the action of theophylline, the maximum response is the same in both cases. This indicates that the amount of lipase has not been changed by treatment with thyroxin. Direct evidence that thyroxin induces the synthesis of adenyl cyclase is shown by experiments in which the enzyme is assayed after administration of thyroxin for 5 days. Figure 4 shows that the increase in the sensitivity of adipose tissue to NE is correlated with the increase in the amount of adenyl cyclase.

The final experiments involve a comparison of adipose tissue from normal and

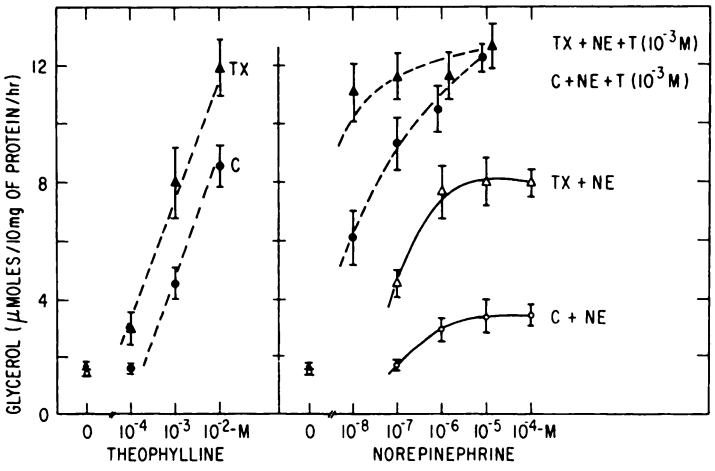


FIG. 3. Left, lipolytic effects of theophylline (T) in adipose tissue of normal and hyperthyroid rats; right, lipolytic effects of NE and of combinations of NE and theophylline (T) in normal and hyperthyroid rats. C, normal rats; TX, *l*-thyroxin — 1 mg/kg s.c. every day for 5 days.

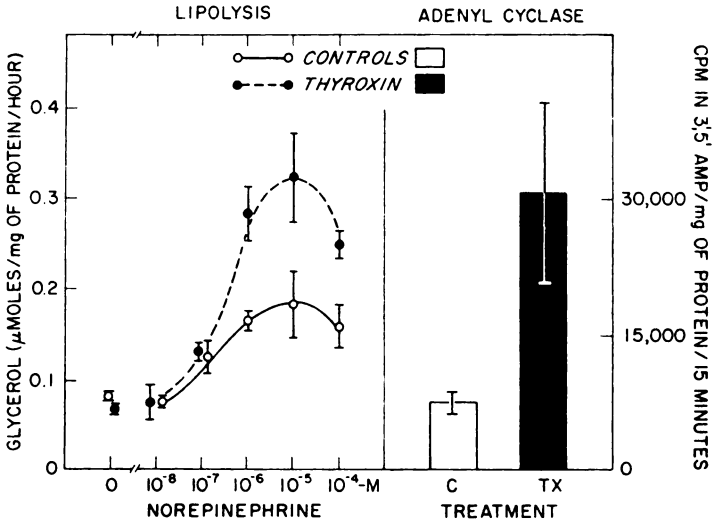


FIG. 4. Left, lipolytic effects of NE in adipose tissue of normal and hyperthyroid rats; right, adenyl cyclase in adipose tissue of normal and hyperthyroid rats. *l*-Thyroxin-1 mg/kg s.c. every day for 5 days; adenyl cyclase was assayed by a method modified from Rabinowitz *et al.* (20) using H^3-ATP as substrate.

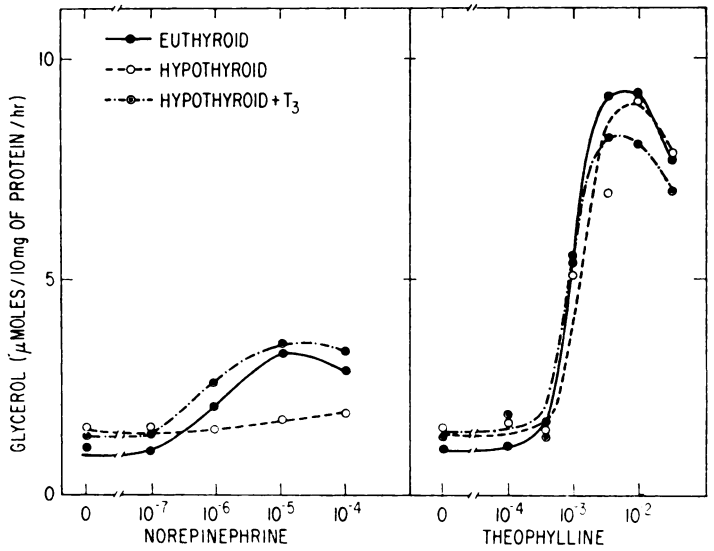


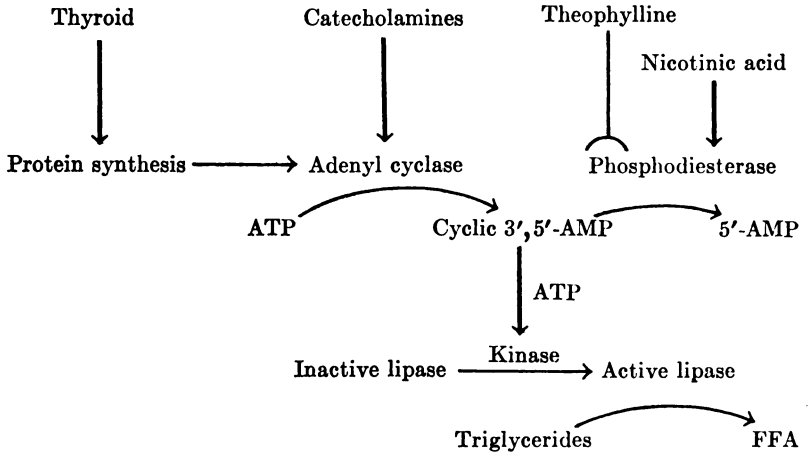
FIG. 5. Left, lipolytic effects of NE in adipose tissue of normal and hypothyroid, and hypothyroid rats treated with triiodothyronine (T_3); right, lipolytic effects of theophylline in adipose tissue of normal and hypothyroid, and hypothyroid rats treated with triiodothyronine (T_3); *l*-triiodothyronine 0.25 mg/kg s.c. 48 hr before sacrifice.

thyroidectomized rats. The glycerol released from the adipose tissue of thyroidectomized rats is increased only slightly by NE; in contrast, when the thyroidectomized animals are given a single dose of triiodothyronine, responsiveness to NE is restored within 48 hr (fig. 5, left). At first glance this action of the thyroid hormone might be interpreted as a permissive one since it only restores the lipolytic response to normal. However, after treatment with the hormone over a longer period of time the response to NE becomes considerably greater than normal. Figure 5 (right) compares the lipolytic effects of various doses of theophylline on adipose tissue from these animals. All the tissues show a maximal lipolysis and this result indicates that after thyroidectomy the amount of lipase has not changed but rather that the amount of adenyl cyclase has decreased.

SUMMARY

Rats whose adrenergic nerve function has been blocked are similar to adrenalectomized rats in their responses to external stimuli that require an increased expenditure of energy. The basal metabolism is almost normal; but on exposure to cold or to muscular work, neither animal can elicit piloerection, vasoconstriction or shivering; nor do they show lipolytic or glycogenolytic responses to epinephrine. The finding that epinephrine fails to restore these responses in adrenalectomized animals indicates that the incapacity of these animals to withstand cold or strenuous work results from failure of sympathetic target organs to respond to transmitted messages. Communications are re-established by aldosterone as well as glucocorticoids, suggesting that the inexcitability of adrenergic receptors after adrenalectomy is related to changes in electrolytes.

An important contribution to biology has been the concept of Sutherland and co-workers that cyclic 3′,5′-AMP is the chemical trigger mediating catecholamine action in sympathetic target organs. These authors have outlined the various steps in the activation of phosphorylase by catecholamines. Some of the steps in the activation of another sympathetic function, the breakdown of triglycerides to FFA, may be outlined as follows:



Catecholamines stimulate lipolysis by causing an instantaneous activation of adenyl cyclase, thereby increasing the steady-state level of cyclic 3′,5′-AMP. This response, however, is limited by the destruction of cyclic 3′,5′-AMP by phosphodiesterase. On the other hand, the steady-state level of cyclic 3′,5′-AMP is increased still further by theophylline, which inhibits phosphodiesterase. As a result, lipase is activated to a much greater extent by theophylline than by the catecholamines.

The effect of catecholamines is inhibited by such diverse compounds as *beta*-adrenergic blocking agents and nicotinic acid. The *beta* blocking agents act by competitively blocking catecholamines at adenyl cyclase, whereas the inhibitory effects of nicotinic acid appear to be due, at least in part, to stimulation of phosphodiesterase. This action would explain why nicotinic acid has such a marked effect on the basal output of FFA. In addition, the effects of catecholamines are prevented by adrenalectomy, presumably through lack of proper ionic environment at the receptor site.

Finally, treatment with thyroid hormone induces the formation of additional adenyl cyclase; in contrast, thyroidectomy reduces the amount of enzyme. In neither case is the amount of lipase affected. Should these effects on the fat pad be typical of other thyroid functions, the interplay between these systems could be explained by a given amount of catecholamine activating a larger amount of adenyl cyclase. As a logical extension of this view, certain actions of thyroid hormones should be diminished after sympathectomy. This might explain why certain sympatholytic agents produce ameliorative effects in patients with hyperthyroidism.

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